```
=> file medline caplus biosis biotechds scisearch embase
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 11:14:30 ON 19 MAR 2003

FILE 'CAPLUS' ENTERED AT 11:14:30 ON 19 MAR 2003
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2003 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 11:14:30 ON 19 MAR 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'BIOTECHDS' ENTERED AT 11:14:30 ON 19 MAR 2003
COPYRIGHT (C) 2003 THOMSON DERWENT AND INSTITUTE FOR SCIENTIFIC INFORMATION

FILE 'SCISEARCH' ENTERED AT 11:14:30 ON 19 MAR 2003
COPYRIGHT (C) 2003 Institute for Scientific Information (ISI) (R)

FILE 'EMBASE' ENTERED AT 11:14:30 ON 19 MAR 2003
COPYRIGHT (C) 2003 Elsevier Science B.V.  All rights reserved.

=> s  9 -18:0-ACP desaturase
L1          24 9 -18:0-ACP DESATURASE

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          10 DUP REM L1 (14 DUPLICATES REMOVED)

=> s l2 and (mutant? or mutation? or variant?)
L3           8 L2 AND (MUTANT? OR MUTATION? OR VARIANT?)

=> s l3 and (114 or 117 or 118 or 179 or 181 or 188)
L4           2 L3 AND (114 OR 117 OR 118 OR 179 OR 181 OR 188)

=> d l4 1-8 ibib ab

L4   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS
ACCESSION NUMBER:         2000:881180  CAPLUS
DOCUMENT NUMBER:          134:52227
TITLE:                    General methods for directed mutagenesis and its
                          application for mutant fatty acid
                          desaturases
INVENTOR(S):              Shanklin, John
PATENT ASSIGNEE(S):       Brookhaven Science Associates, Llc, USA
SOURCE:                   PCT Int. Appl., 53 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   3
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
     ---------------   ----  --------       ---------------   --------
     WO 2000075170      A1   20001214       WO 2000-US15741   20000608
         W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,
             CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
             IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
             MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI,
             SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ,
             BY, KG, KZ, MD, RU, TJ, TM
```

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1214334 A1 20020619 EP 2000-939675 20000608

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.: US 1999-328550 A 19990609
WO 2000-US15741 W 20000608

AB The present invention relates to a method for producing **mutants** of a fatty acid desaturase, the unmutagenized precursor having 18-carbon chain substrate specificity, the produced **mutants** having substantially increased activity towards fatty acid substrates with chains contg. fewer than 18 carbons, relative to the activity of unmutagenized precursor desaturase. **Mutations** in the coding region of castor .DELTA.**9-18:0-ACP** **desaturase** which result in amino acid substitutions at position **114, 117, 118, 179, 181,** or **188,** and combined substitutions at positions **114** and **188,** are described. A preferred bacterial strain used in the selection system, Escherichia coli MH13, is an unsatd. fatty auxotroph requiring exogenous unsatd. fatty acids to proliferate. Altered fatty acid compns. are demonstrated in transgenic Escherichia coli and Arabidopsis thaliana. DNA encoding the **mutants,** host cells contg. the DNA, transgenic plants expressing the DNA, and the desaturase enzyme produced by the method are also recited. Combinatorial full positional randomization of codons can be accomplished by a variety of methods. One such method is the use of overlap-extension PCR to replace all codons for candidate position amino acids with NNK or NNN. The process of overlap-extension PCR has been used to simultaneously introduce at least 9 independent **mutations** into a particular coding sequence. In another embodiment, a subset of one or more of the candidate positions are incompletely randomized, while the other candidate positions are fully randomized.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:395727 CAPLUS

DOCUMENT NUMBER: 129:146255

TITLE: A determinant of substrate specificity predicted from the acyl-acyl carrier protein desaturase of developing cat's claw seed

AUTHOR(S): Cahoon, Edgar B.; Shah, Salehuzzaman; Shanklin, John; Browse, John

CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, NY, 11976, USA

SOURCE: Plant Physiology (1998), 117(2), 593-598
CODEN: PLPHAY; ISSN: 0032-0889

PUBLISHER: American Society of Plant Physiologists

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cat's claw (Doxantha unguis-cati L.) vine accumulates nearly 80% palmitoleic acid (16:1.DELTA.9) plus cis-vaccenic acid (18:1.DELTA.11) in its seed oil. To characterize the biosynthetic origin of these unusual fatty acids, cDNAs for acyl-acyl carrier protein (acyl-ACP) desaturases were isolated from developing cat's claw seeds. The predominant acyl-ACP desaturase cDNA identified encoded a polypeptide that is closely related to the stearoyl (.DELTA.**9-18:0**)**-ACP** **desaturase** from castor (Ricinis communis L.) and other species. Upon expression in Escherichia coli, the cat's claw polypeptide functioned as a .DELTA.9 acyl-ACP desaturase but displayed a distinct substrate specificity for palmitate (16:0)-ACP rather than stearate (18:0)-ACP. Comparison of the predicted amino acid sequence of the cat's claw enzyme

with that of the castor .DELTA.**9-18:0-ACP desaturase** suggested that a single amino acid substitution (L118W) might account in large part for the differences in substrate specificity between the two desaturases. Consistent with this prediction, conversion of leucine-**118** to tryptophan in the mature castor .DELTA.**9-18:0-ACP desaturase** resulted in an 80-fold increase in the relative specificity of this enzyme for 16:0-ACP. The alteration in substrate specificity obsd. in the L118W **mutant** is in agreement with a crystallog. model of the proposed substrate-binding pocket of the castor .DELTA.**9-18:0-ACP desaturase**.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l4 1-8 ibib ab

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:881180 CAPLUS
DOCUMENT NUMBER: 134:52227
TITLE: General methods for directed mutagenesis and its application for **mutant** fatty acid desaturases
INVENTOR(S): Shanklin, John
PATENT ASSIGNEE(S): Brookhaven Science Associates, Llc, USA
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000075170	A1	20001214	WO 2000-US15741	20000608
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1214334	A1	20020619	EP 2000-939675	20000608
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				

PRIORITY APPLN. INFO.: US 1999-328550 A 19990609
WO 2000-US15741 W 20000608

AB The present invention relates to a method for producing **mutants** of a fatty acid desaturase, the unmutagenized precursor having 18-carbon chain substrate specificity, the produced **mutants** having substantially increased activity towards fatty acid substrates with chains contg. fewer than 18 carbons, relative to the activity of unmutagenized precursor desaturase. **Mutations** in the coding region of castor .DELTA.**9-18:0-ACP desaturase** which result in amino acid substitutions at position **114, 117, 118, 179, 181,** or **188**, and combined substitutions at positions **114** and **188**, are described. A preferred bacterial strain used in the selection system, Escherichia coli MH13, is an unsatd. fatty auxotroph requiring exogenous unsatd. fatty acids to proliferate. Altered fatty

acid compns. are demonstrated in transgenic Escherichia coli and Arabidopsis thaliana. DNA encoding the **mutants**, host cells contg. the DNA, transgenic plants expressing the DNA, and the desaturase enzyme produced by the method are also recited. Combinatorial full positional randomization of codons can be accomplished by a variety of methods. One such method is the use of overlap-extension PCR to replace all codons for candidate position amino acids with NNK or NNN. The process of overlap-extension PCR has been used to simultaneously introduce at least 9 independent **mutations** into a particular coding sequence. In another embodiment, a subset of one or more of the candidate positions are incompletely randomized, while the other candidate positions are fully randomized.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:395727 CAPLUS

DOCUMENT NUMBER: 129:146255

TITLE: A determinant of substrate specificity predicted from the acyl-acyl carrier protein desaturase of developing cat's claw seed

AUTHOR(S): Cahoon, Edgar B.; Shah, Salehuzzaman; Shanklin, John; Browse, John

CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, NY, 11976, USA

SOURCE: Plant Physiology (1998), 117(2), 593-598
CODEN: PLPHAY; ISSN: 0032-0889

PUBLISHER: American Society of Plant Physiologists

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cat's claw (Doxantha unguis-cati L.) vine accumulates nearly 80% palmitoleic acid (16:1.DELTA.9) plus cis-vaccenic acid (18:1.DELTA.11) in its seed oil. To characterize the biosynthetic origin of these unusual fatty acids, cDNAs for acyl-acyl carrier protein (acyl-ACP) desaturases were isolated from developing cat's claw seeds. The predominant acyl-ACP desaturase cDNA identified encoded a polypeptide that is closely related to the stearoyl (.DELTA.**9**-**18**:**0**)-**ACP** **desaturase** from castor (Ricinis communis L.) and other species. Upon expression in Escherichia coli, the cat's claw polypeptide functioned as a .DELTA.9 acyl-ACP desaturase but displayed a distinct substrate specificity for palmitate (16:0)-ACP rather than stearate (18:0)-ACP. Comparison of the predicted amino acid sequence of the cat's claw enzyme with that of the castor .DELTA.**9**-**18**:**0**-**ACP** **desaturase** suggested that a single amino acid substitution (L118W) might account in large part for the differences in substrate specificity between the two desaturases. Consistent with this prediction, conversion of leucine-**118** to tryptophan in the mature castor .DELTA.**9**-**18**:**0**-**ACP** **desaturase** resulted in an 80-fold increase in the relative specificity of this enzyme for 16:0-ACP. The alteration in substrate specificity obsd. in the L118W **mutant** is in agreement with a crystallog. model of the proposed substrate-binding pocket of the castor .DELTA.**9**-**18**:**0**-**ACP** **desaturase**.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d 14 3-8 ibib ab
2 ANSWERS ARE AVAILABLE. SPECIFIED ANSWER NUMBER EXCEEDS ANSWER SET SIZE
The answer numbers requested are not in the answer set.
ENTER ANSWER NUMBER OR RANGE (1):d 14 3 ibib ab
ANSWER NUMBERS NOT CORRECTLY SPECIFIED

```
Enter an answer number,        Example: 10
several answer numbers,        Example: 3,7,10
a range of answer numbers,     Example: 5-10
or a combination of these.     Example: 3,7,9-10,15
ENTER ANSWER NUMBER OR RANGE (1):end

=> d his

     (FILE 'HOME' ENTERED AT 11:13:45 ON 19 MAR 2003)

     FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT
     11:14:30 ON 19 MAR 2003
L1            24 S  9 -18:0-ACP DESATURASE
L2            10 DUP REM L1 (14 DUPLICATES REMOVED)
L3             8 S L2 AND (MUTANT? OR MUTATION? OR VARIANT?)
L4             2 S L3 AND (114 OR 117 OR 118 OR 179 OR 181 OR 188)

=> d l3 1-8 ibib ab

L3   ANSWER 1 OF 8     MEDLINE
ACCESSION NUMBER:   2001331977     MEDLINE
DOCUMENT NUMBER:    21293111   PubMed ID: 11294879
TITLE:              Engineering delta 9-16:0-acyl carrier protein (ACP)
                    desaturase specificity based on combinatorial saturation
                    mutagenesis and logical redesign of the castor delta
                    9-18:0-ACP
                    desaturase.
AUTHOR:             Whittle E; Shanklin J
CORPORATE SOURCE:   Biology Department, Brookhaven National Laboratory, Upton,
                    New York 11973, USA.
SOURCE:             JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Jun 15) 276 (24)
                    21500-5.
                    Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:       United States
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200107
ENTRY DATE:         Entered STN: 20010723
                    Last Updated on STN: 20030105
                    Entered Medline: 20010719
AB   Six amino acid locations in the soluble castor Delta(9)-18:0-acyl carrier
     protein (ACP) desaturase were identified that can affect substrate
     specificity. Combinatorial saturation mutagenesis of these six amino
     acids, in conjunction with selection, using an unsaturated fatty acid
     auxotroph system, led to the isolation of variants with up to
     15-fold increased specific activity toward 16-carbon substrates. The most
     improved mutant, com2, contained two substitutions (T117R/G188L)
     common to five of the 19 complementing variants subjected to
     further analysis. These changes, when engineered into otherwise wild-type
     18:0-ACP desaturase to make mutant 5.2, produced a 35-fold
     increase in specific activity with respect to 16-carbon substrates.
     Kinetic analysis revealed changes in both k(cat) and K(m) that result in
     an 82-fold improvement in specificity factor for 16-carbon substrate
     compared with wild-type enzyme. Improved substrate orientation apparently
     compensated for loss of binding energy that results from the loss of
     desolvation energy for 16-carbon substrates. Mutant 5.2 had
     specific activity for 16-carbon substrates 2 orders of magnitude higher
     than those of known natural 16-carbon specific desaturases. These data
     support the hypothesis that it should be possible to reengineer archetypal
     enzymes to achieve substrate specificities characteristic of recently
     evolved enzymes while retaining the desired stability and/or turnover
     characteristics of a parental paralog.
```

L3 ANSWER 2 OF 8 MEDLINE
ACCESSION NUMBER: 2001039056 MEDLINE
DOCUMENT NUMBER: 20504514 PubMed ID: 11027301
TITLE: Substrate-dependent **mutant** complementation to select fatty acid desaturase **variants** for metabolic engineering of plant seed oils.
AUTHOR: Cahoon E B; Shanklin J
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2000 Oct 24) 97 (22) 12350-5. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001128

AB We demonstrate that naturally occurring C(14) and C(16)-specific acyl-acyl carrier protein (ACP) desaturases from plants can complement the unsaturated fatty acid (UFA) auxotrophy of an Escherichia coli fabA/fadR **mutant**. Under the same growth conditions, C(18)-specific delta(9)-stearoyl (18:0)-ACP desaturases are unable to complement the UFA auxotrophy. This difference most likely results from the presence of sufficient substrate pools of C(14) and C(16) acyl-ACPs but a relative lack of C(18) acyl-ACP pools in E. coli to support the activities of the plant fatty acid desaturase. Based on this, a substrate-dependent selection system was devised with the use of the E. coli UFA auxotroph to isolate **mutants** of the castor delta(**9**)-**18**:**0-ACP desaturase** that display enhanced specificity for C(14) and C(16) acyl-ACPs. Using this selection system, a number of desaturase **variants** with altered substrate specificities were isolated from pools of randomized **mutants**. These included several G188L **mutant** isolates, which displayed a 15-fold increase in specific activity with 16:0-ACP relative to the wild-type castor delta(**9**)-**18**:**0-ACP desaturase**. Expression of this **mutant** in Arabidopsis thaliana resulted in the accumulation of unusual monounsaturated fatty acids to amounts of >25% of the seed oil. The bacterial selection system described here thus provides a rapid means of isolating **variant** fatty acid desaturase activities for modification of seed oil composition.

L3 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:586274 CAPLUS
DOCUMENT NUMBER: 137:307376
TITLE: What limits production of unusual monoenoic fatty acids in transgenic plants?
AUTHOR(S): Suh, Mi Chung; Schultz, David J.; Ohlrogge, John B.
CORPORATE SOURCE: Department of Plant Biology, Michigan State University, East Lansing, MI, 48824, USA
SOURCE: Planta (2002), 215(4), 584-595
CODEN: PLANAB; ISSN: 0032-0935
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Unusual monounsatd. fatty acids are major constituents (greater than 80%) in seeds of Coriandrum sativum L. (coriander) and Thunbergia alata Bojer, as well as in glandular trichomes (greater than 80% derived products) of Pelargonium .times.hortorum (geranium). These diverged fatty acid structures are produced via distinct plastidial acyl-acyl carrier protein

(ACP) desaturases. When expressed in Arabidopsis thaliana (L.) Heynh. under strong seed-specific promoters the unusual acyl-ACP desaturases resulted in accumulation of unusual monoene fatty acids at 1-15% of seed fatty acid mass. In this study, we have examd. several factors that potentially limit higher prodn. of unusual monoenes in transgenic oilseeds. (i) Immunoblots indicated that the introduced desaturases were expressed at levels equiv. to or higher than the endogenous .DELTA.**9 18:0-ACP desaturase**. However, the level of unusual fatty acid produced in transgenic plants was not correlated with the level of desaturase expression. (ii) The unusual desaturases were expressed in several backgrounds, including antisense 18:0-ACP desaturase plants, in fabl **mutants**, and co-expressed with specialized ACP or ferredoxin isoforms. None of these expts. led to high prodn. of expected products. (iii) No evidence was found for degrdn. of the unusual fatty acids during seed development. (iv) Petroselinic acid added to developing seeds was incorporated into triacylglycerol as readily as oleic acid, suggesting no major barriers to its metab. by enzymes of glycerolipid assembly. (v) In vitro and in situ assay of acyl-ACP desaturases revealed a large discrepancy of activity when comparing unusual acyl-ACP desaturases with the endogenous .DELTA.**9 18:0-ACP desaturase**. The combined results, coupled with the sensitivity of acyl-ACP desaturase activity to centrifugation and low salt or detergent suggests low prodn. of unusual monoenes in transgenic plants may be due to the lack of, or incorrect assemble of, a necessary multi-component enzyme assocn.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:881180 CAPLUS
DOCUMENT NUMBER: 134:52227
TITLE: General methods for directed mutagenesis and its application for **mutant** fatty acid desaturases
INVENTOR(S): Shanklin, John
PATENT ASSIGNEE(S): Brookhaven Science Associates, Llc, USA
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000075170	A1	20001214	WO 2000-US15741	20000608
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1214334	A1	20020619	EP 2000-939675	20000608
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				

PRIORITY APPLN. INFO.: US 1999-328550 A 19990609
WO 2000-US15741 W 20000608

AB The present invention relates to a method for producing **mutants** of a fatty acid desaturase, the unmutagenized precursor having 18-carbon chain substrate specificity, the produced **mutants** having

substantially increased activity towards fatty acid substrates with chains contg. fewer than 18 carbons, relative to the activity of unmutagenized precursor desaturase. **Mutations** in the coding region of castor .DELTA.**9-18:0-ACP** **desaturase** which result in amino acid substitutions at position 114, 117, 118, 179, 181, or 188, and combined substitutions at positions 114 and 188, are described. A preferred bacterial strain used in the selection system, Escherichia coli MH13, is an unsatd. fatty auxotroph requiring exogenous unsatd. fatty acids to proliferate. Altered fatty acid compns. are demonstrated in transgenic Escherichia coli and Arabidopsis thaliana. DNA encoding the **mutants**, host cells contg. the DNA, transgenic plants expressing the DNA, and the desaturase enzyme produced by the method are also recited. Combinatorial full positional randomization of codons can be accomplished by a variety of methods. One such method is the use of overlap-extension PCR to replace all codons for candidate position amino acids with NNK or NNN. The process of overlap-extension PCR has been used to simultaneously introduce at least 9 independent **mutations** into a particular coding sequence. In another embodiment, a subset of one or more of the candidate positions are incompletely randomized, while the other candidate positions are fully randomized.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:759826 CAPLUS
DOCUMENT NUMBER: 134:39564
TITLE: Stearoyl-acyl carrier protein and unusual acyl-acyl carrier protein desaturase activities are differentially influenced by ferredoxin
AUTHOR(S): Schultz, David J.; Suh, Mi Chung; Ohlrogge, John B.
CORPORATE SOURCE: Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824, USA
SOURCE: Plant Physiology (2000), 124(2), 681-692
CODEN: PLPHAY; ISSN: 0032-0889
PUBLISHER: American Society of Plant Physiologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Acyl-acyl carrier protein (ACP) desaturases function to position a single double bond into an acyl-ACP substrate and are best represented by the ubiquitous .DELTA.**9 18:0-ACP** **desaturase**. Several **variant** acyl-ACP desaturases have also been identified from species that produce unusual monoenoic fatty acids. All known acyl-ACP desaturase enzymes use ferredoxin as the electron-donating cofactor, and in almost all previous studies the photosynthetic form of ferredoxin rather than the non-photosynthetic form has been used to assess activity. The influence of different forms of ferredoxin on acyl-ACP desaturases was examd. Using combinations of in vitro acyl-ACP desaturase assays and [14C]malonyl-CoA labeling studies, it was detd. that heterotrophic ferredoxin isoforms support up to 20-fold higher unusual acyl-ACP desaturase activity in coriander (Coriandrum sativum), Thunbergia alata, and garden geranium (Pelargonium .times. hortorum) when compared with photosynthetic ferredoxin isoforms. Heterotrophic ferredoxin also increases activity of the ubiquitous .DELTA.**9 18:0-ACP desaturase** 1.5-3.0-fold in both seed and leaf exts. These results suggest that ferredoxin isoforms may specifically interact with acyl-ACP desaturases to achieve optimal enzyme activity and that heterotrophic isoforms of ferredoxin may be the in vivo electron donor for this reaction.

REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:395727 CAPLUS
DOCUMENT NUMBER: 129:146255
TITLE: A determinant of substrate specificity predicted from the acyl-acyl carrier protein desaturase of developing cat's claw seed
AUTHOR(S): Cahoon, Edgar B.; Shah, Salehuzzaman; Shanklin, John; Browse, John
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, NY, 11976, USA
SOURCE: Plant Physiology (1998), 117(2), 593-598
CODEN: PLPHAY; ISSN: 0032-0889
PUBLISHER: American Society of Plant Physiologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cat's claw (Doxantha unguis-cati L.) vine accumulates nearly 80% palmitoleic acid (16:1.DELTA.9) plus cis-vaccenic acid (18:1.DELTA.11) in its seed oil. To characterize the biosynthetic origin of these unusual fatty acids, cDNAs for acyl-acyl carrier protein (acyl-ACP) desaturases were isolated from developing cat's claw seeds. The predominant acyl-ACP desaturase cDNA identified encoded a polypeptide that is closely related to the stearoyl (.DELTA.**9-18:0**)-**ACP** **desaturase** from castor (Ricinis communis L.) and other species. Upon expression in Escherichia coli, the cat's claw polypeptide functioned as a .DELTA.9 acyl-ACP desaturase but displayed a distinct substrate specificity for palmitate (16:0)-ACP rather than stearate (18:0)-ACP. Comparison of the predicted amino acid sequence of the cat's claw enzyme with that of the castor .DELTA.**9-18:0-ACP** **desaturase** suggested that a single amino acid substitution (L118W) might account in large part for the differences in substrate specificity between the two desaturases. Consistent with this prediction, conversion of leucine-118 to tryptophan in the mature castor .DELTA.**9-18:0-ACP** **desaturase** resulted in an 80-fold increase in the relative specificity of this enzyme for 16:0-ACP. The alteration in substrate specificity obsd. in the L118W **mutant** is in agreement with a crystallog. model of the proposed substrate-binding pocket of the castor .DELTA.**9-18:0-ACP** **desaturase**.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:333116 CAPLUS
DOCUMENT NUMBER: 127:46988
TITLE: Redesign of soluble fatty acid desaturases from plants from altered substrate specificity and double bond position
AUTHOR(S): Cahoon, Edgar B.; Lindqvist, Ylva; Schneider, Gunter; Shanklin, John
CORPORATE SOURCE: Biol. Dep., Brookhaven Natl. Lab., Upton, NY, 11973, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1997), 94(10), 4872-4877
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Acyl-acyl carrier protein (ACP) desaturases introduce double bonds at specific positions in fatty acids of defined chain lengths and are one of the major determinants of the monounsatd. fatty acid compn. of vegetable oils. Mutagenesis studies were conducted to det. the structural basis for the substrate and double bond positional specificities displayed by acyl-ACP desaturases. By replacement of specific amino acid residues in a

.DELTA.6-palmitoyl (16:0)-ACP desaturase with their equiv. from a .DELTA.9-stearoyl (18:0)-ACP desaturase, **mutant** enzymes were identified that have altered fatty acid chain-length specificities or that can insert double bonds into either the .DELTA.6 or .DELTA.9 positions of 16:0- and 18:0-ACP. Most notably, by replacement of 5 amino acids (A181T/A200F/S205N/L206T/G207A), the .DELTA.6-16:0-AcP desaturase was converted into an enzyme that functions principally as a .DELTA.**9-18:0-ACP desaturase**. Many of the determinants of fatty acid chain-length specificity in these **mutants** are found in residues that line the substrate binding channel as revealed by x-ray crystallog. of the .DELTA.**9-18:0-ACP desaturase**. The crystallog. model of the active site is also consistent with the diverged activities assocd. with naturally occurring **variant** acyl-ACP desaturases. In addn., on the basis of the active-site model, a .DELTA.**9-18:0-AcP desaturase** was converted into an enzyme with substrate preference for 16:0-ACP by replacement of 2 residues (L118F/P179I). These results demonstrate the ability to rationally modify acyl-ACP desaturase activities through site-directed mutagenesis and represent a first step toward the design of acyl-AcP desaturases for the prodn. of novel monounsatd. fatty acids in transgenic oilseed crops.

L3 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1997:330585 CAPLUS
DOCUMENT NUMBER: 127:46978
TITLE: Characterization of a structurally and functionally diverged acyl-acyl carrier protein desaturase from milkweed seed
AUTHOR(S): Cahoon, Edgar B.; Coughlan, Sean J.; Shanklin, John
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, NY, 11973, USA
SOURCE: Plant Molecular Biology (1997), 33(6), 1105-1110
CODEN: PMBIDB; ISSN: 0167-4412
PUBLISHER: Kluwer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A cDNA for a structurally **variant** acyl-acyl carrier protein (ACP) desaturase was isolated from milkweed (Asclepias syriaca) seed, a tissue enriched in palmitoleic (16:1.DELTA.9) and cis-vaccenic (18:1.DELTA.11) acids. Exts. of Escherichia coli that express the milkweed cDNA catalyzed .DELTA.9 desatn. of acyl-ACP substrates, and the recombinant enzyme exhibited seven- to ten-fold greater specificity for palmitoyl (16:0)-ACP and 30-fold greater specificity for myristoyl (14:0)-ACP than did known .DELTA.9-stearoyl (18:0)-ACP desaturases. Like other **variant** acyl-ACP desaturases reported to date, the milkweed enzyme contains fewer amino acids near its N-terminus compared to previously characterized .DELTA.9-18:0-ACP desaturases. Based on the activity of an N-terminal deletion **mutant** of a .DELTA.**9-18:0-ACP desaturase**, this structural feature likely does not account for differences in substrate specificities.

=> d his

(FILE 'HOME' ENTERED AT 11:13:45 ON 19 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT 11:14:30 ON 19 MAR 2003
L1 24 S 9 -18:0-ACP DESATURASE
L2 10 DUP REM L1 (14 DUPLICATES REMOVED)
L3 8 S L2 AND (MUTANT? OR MUTATION? OR VARIANT?)

L4 2 S L3 AND (114 OR 117 OR 118 OR 179 OR 181 OR 188)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	57.20	57.41

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-6.51	-6.51

STN INTERNATIONAL LOGOFF AT 11:20:22 ON 19 MAR 2003